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FAVORABLE INFLUENCE OF THE INFLUENZAL VIRUS ON ASSOCIATED INFECTIONS

P. Bordet and L. Quersin-Thiry

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Paul Bordet ant Lise Quersin-Thiry. Extrait des Annales de L'Institut Pasteur, Avril 1953, Vol. 84, pp. 1-8.
Tanslated by Bernice MacDonald.

In a previous article (1) we have described the effect of intraperitoneal inoculation of influenzal virus on the guinea pig, placing in evidence the increase of receptivity which this inoculation determines in regard to the Pfeiffer bacillus introduced the same way. Let us recall briefly that the injection of 20,000 hemagglutinant units make an abundant and fluid exudate appear regularly coming out under pressure when the peritoneum is punctures 24 to 48 hours after inoculation, and which, under microscopic examination, is characterized by the great frequency of leucocytic pycnosis images. Observed in the peritoneal cavity, a very asceptic milieu, of an animal not receptive to the influenzal infection and in which the virus does not multiply, the effects of nature manifestly toxic contribute to clarify the physiopathology of influenza. They seemingly allow, in particular, the explanation for the only toxcicity of the virus the flashing forms of influenza which has been observed in the course of the epedemic of 1918-1919 and which, in the autopsy showed the lungs full of serous exudate. The experiments related in this preceding articleshowed that the guinea pig having re-

ceived such a dise manifest, in regard to the Pfeiffer bacillus introduced on half hour later in the form of culture on blood bouillon aged 24hours, a sensitivity 4 to 6 times grater than that of the test.

Following this research we have recorded the new results which we record in this second article.

From the very first we have stated that the differences in receptivity to the Pfeiffer bacillus, between tes guinea pigs and the one inoculated with influenzal virus, appear very much more notable than in our previous experiments, if instead of using the cultire of that bacteria on glood-bouillon, one inoculates in the positive of culture on solid media.

The strain of Pfeiffer bacillus used is the same as that used in previous tests. In view of its inoculation, 24 hour cultures on continuous beds on blood-gelose are diluted after elemination of bottom liquid, in the bouillon diluted to 1.20 in physiological solution. The guineapigs used always weighed between 300 and 325 gr. The eluat of virus, titrating most often 8,000 hemagglutinat units, per ml., is injected at the rate of 20,000 units, are usually under a volume of 25 ml. The guinea pigs before serving as tests receive the same volume of physiological solution added to the product of laquage of hematites which give it a rose color at leat eqaul to that of the virulent eluat. We do not recall her the method of preparing this eluat: it has been described in our preceding article.

According to our tests, which have yielded 33 animals,

the fatal dose of Pfeiffer bacillus for the test guinea pigs is located around ½ of culture on blood-gelose, dose which kills half of the number of test guinea pigs (out of 6); death regularly follows the injection of a dose greater than ½ culture, on the other hand, the injection of 1/8 of the culture has killed 2 out of 7 guinea pigs; and the injection of 1.16 of culture, or a lesser dose, always has been followed by recovery. In the guinea pig inoculated ½ an hour before with eluted virus (20,000 units), the fatal dose, in our experiments which have carried up to 42 animals, was often less than 1/1000 of culture, dose which killed 2/3 of the guinea pigs (6 out of 9). In reality, this proportion of 2 out of 3 deaths is verified for all the guinea pigs (26) which after inoculation of influenzal virus, have received the doses of Pfeiffer bacillus comprising between 1/250 and 1/4000 of culture; the mortality is constant for these very high doses. The injection of 1.1000 of culture is therfore often fatal for the guinea pig inocualted with influenzal virus althoughit is only ½ of culture for the test guinea pig. One makes sure, certainly, for other tests, that the dose of virus inoculated (20,000 units) is regularly well tolerated, that this has already been established in our previous article, where we have shown that that does not represent the 1/8 part of the fatal dose. Finally in the animals which die after inoculation of the Pfeiffer bacillus- death usually follows between 15 and 30 hours later- the generalization of the infection by this bacteria has been regularly confirmed by inocualtion of heart blood on blood-gelose.

Therefore one can estimate on the average of 250, at least, the coefficient of increase, under the action of the influenzal virus, of the receptivity to the Pfeiffer bacillus; this coefficient occassionally attains 2,000. This variation observed using the Pfeiffer bacillus on solid media is therfore considerably higher to those (4 to 6) which we have indicated in our previous tests, allow the use of culture in blood-bouillon. Possibly this difference is due to the fact that the cultures in liquid media conatian the toxic properties which the action tends to disguise the one ascribable to the virulence of the germ. However that may be, it is evident that the use of cultures on solid media imitate the best conditions of natural contamination; also, the observations to which it has given place seem to furnish an experimantal reproduction particularly true of the favorable influence which ithe influenza exerts, in man, on the infection by the Pfeiffer bacillus.

The considerable variation of sensitivity between the test guinea pigs and the influenza guinea pigs allows the estimation, whichwe could not do in previous tests, of the influence of lesser doses of influenzal viruses on the receptivity to the Pfeiffer bacillus.

One injects 1/2000 of culture of the Pfeifferbacillus on blood gelose- or 1/50 of the fatal dose for the test animal- in the peritoneum of the guinea pigs having received half an hour before, the influenzal virus corresponding in quantity to 1,250, 2,500, 5,000 and 10.000 hemagglutinat units while a guinea pig having received 20,000 units is inoculated with 1.1000 of culture of Pfeiffer bacillus.

The sesitivity to the Pfeiffer bacillus therefore deminishes

rapidly when the dose of virus inoculates is reduced.; if the latter does not exceed 5,000 units the guineapigs, in effect, resist the injection of 1.200 of bacterial culture. Nevertheless, we have stated that, the guinea pig dies upon injection of 1/50 of culture of Pfeiffer bacillus and therefore manifests again a greater sensitivity which is tem times that of the test. In this respect, a striking parallelism is ascertained between the degree of sensitivty to the bacterial infection and the importance of toxic effects caused by the virus and which we have described in our previous article: the frequency of leucocytic pycnosis images are not clearly marked after the injection of a dose corresponding to at least 5,000 units. and the abundance ofthe peritoneal exudate diminished rapidly with the quantity of virus inoculated; the semsitization to the Pfeiffer bacillus is therefore not clearly marked except when the toxic effects of the virus are apparent.

Again we point out that the increase of sensitivity to the infection by the Pfeiffer bacillus is attested not only when the virus is inoculated shortly before the bacteria, but also if it is an hour, and the same five hours afterwards: the sensitization action of the virus therefore appears rapid because it declares itself when the virus is inoculated only several hours after the bacteria.

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The importance of the vafiation which separates the fatal doses of influenzal virus and the Pfeiffer bacillus according to whether the virus and the bacteria are used concomitanly

or separately indicates that when the association of the 2 germs entail the death of the animal the latter is not due to a simple accumulation of their respective pathogenic actions but is the resultof a sensitization of the organism, on the toxic action of the virus, to the infection by the bacteria. But it is evidently advisable for research if this sensitization manifests itself specially in regard to the Pfeiffer bacillus or if its is also observed in respect to any pathogenic germs do not behave as associated to the progress of the human influenza.

We have had recourse, for this purpose, to colon bacillus, whose intraperitoneal inoculation of the guinea pig easily causes in this animal a fatal seprecemia. But, in operating under identical conditions to those described below in regard to the Pfeiffer bacillus, we have observed, under the toxic action of the influenzal virus, no increase of sensitivity to infection by the colon bacillus, when the minimum fatal dose for the strain we have used (E. colis VA), is about 1/20 culture on gelose. Because it concerns cholera vibro (Inaba strain) which we have tested equally in this regard, the minimum fatal dose for the guinea pig inoculated with influenzal virus is $\frac{1}{2}$ of culture on gelose, appears about equal to $\frac{1}{2}$ of that fatal dose (1 culture) for the test animal. But the inoculation of heart blood in the animal having died by the inoculation of vibro delay sterile material.: the slight difference of sensitivity observed cannot therefore be considered as showing a favorable influence of the virus on the

cholera infection and it is explained probably by the accumulation in the "influenza" animal, of the toxic effects of the virus and the vibro.

We have on the other hand extended our research to 2 others bacteria, frequently responsible for complications in the course of human influenza, the pneumococci and the streptococci.

Harford, Leidler and Hara (2) have recognized that after inoculation with influenza virus the respiratory way, the inhalation of pneumococci causes in the mice a very serious infection which in the absence of the virus had already caused lesions microscopically discernable from the time the pneumococci were introduced.

Although, in the technique which we use, the inoculation of the bacteria follows by only ½ hour that of the virus, we have observed aclear difference of sensitivity to he pneumococci infection, among the guinea pigs inoculated with virus and the testes guinda pigs. For the strain wehave employed the fatal dose of the test animal is of 1 whole culture on blood-gelose, it is 1/10 of culture for the guinea pig previously inoculated with influenzal virus. The variation of sensitivity os therefore 1 to 10, although it is very appreciable it is therefore much less than that ascertained for the Pfeiffer bacillus.

According to Carlisle (3), intanasal inoculation in mice of an infra-fatal dose of influenzal virus A weakens the resistance of the animal to a hemolytic streptococci of group C subsequently intoduced the same way, it is from 4 to 12 hours

ater the virulent inocualtion that the sensitivity to the streptococci is marked the best; at this moment the fatal dose, according to the author would be 10 times less than in the test mice.

We have used a strain of hemolytic streptococci recently isolated from an anginose exudate and which in the peritoneal cavity of the guinea pig have strongly encysted (sacculated) shapes. In the test guinea pig, the minimum fatal dose is ½ of 24 hour culture on blood-gelose; death follows in 18 to 24 hours and the germ can be seen in the heart by inoculation of blood-gelose. Operating under identical conditions to those described below in regard to germs already studied we have ascertained that after inoculation with 20,000 units of influenzal virus, the fatal dose of the streptococci is found to drop to 1/400 or 1/800 of culture. The coefficient of increase of receptivity under the toxic action of the influenzal virus is therefore between 100 and 200. In addition the "influenza" guinea pig dies occassionally of inoculation of doses weaker than streptococci: 1/6400 of culture in one case.

It is therefore in regard to the Pfeiffer bacillus and the hemolytic streptocccci that the sensititzation, under the toxic action of the influenzal virus proves the stro ngest; well marked with respect to the pneumococci it is nil with regard to the colon bacillus.

Andre Govaerts actually tested in our laboratory if other viruses possessed certain properties of the influenzal virus,

such as the mumps and that of the Newcastle disease, equally divided with it the favorable influences described below.

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On the other hand we have chosen the study to which we have alluded briefly in our preceding articel, of the possible ratio uniting, to the infectious power and the hemagglutinat power, the properties of virus responsible for the toxic effects which we ha have described. It has been indicated to us that like G. Henle and W. Henle (4) have pointed out, the toxic power is a little more sensitive to heat than the infectious power, but this difference in sensitivity is slight and difficult to prove, the toxic properties concerned in our experiments are completely abolished by heating for 30 minutes at 58°. On the other hand, the independence between these properties and the hemagglutinat power, a little more resistant to heat, seem to us to established by ther experiment that follows.

A portion of virulent eluat obtained is heated for an hour at 58° in a buffered physiologic solution and the hemagglutination titer is equal to 1/8192. This heating is to reduce the titer to ½ of its initial value, that is to 1/2048. But the intra-peritonela injection of 10 ml. of this eluat does not produce any of the toxic effects we have described, whereas the latter appear with their usual characteristics, after injection of an equal volume of the same eluat used cold, but previously diluted to ½ and whose hemagglutinat titer is equal to the

heated eluat. Various other results, in addition contribute to demonstrate the independence between the toxic powers amd the hemagglutinat power. We point out that a virulent suspension deprived of its enzymatic power by heating to 56° and whose hemagglutinat titer is 1/2048, does not lose any of its capacity to release its toxic powers, if by previousmixing to an equal volume of ovomucinne(1) its titer has been lowered to 1/16 whereas of course the injection of the same virulent suspension lowered the titer to 1/16 by dilutions, remains without effect.

Also we point out that the injection of 5 ml. of cholera filtrate (2) rich in enzyme RDE, done an hour before that of a virulent eluay does not affect any of the toxic effects of the latter.

We conclude by a few complimentary pieces of information collected in the course of our research. We have observed that the virus delay suited to produce the toxic effects when one inocu= lates it adsorbed on red corpuscels, which is not astonishing because, as one knows, the virus absorbed elutes itself at 37°. It is very interesting that the toxic effects are equally established when the virus injected is adsorbed on aluminum hydrate like the following experiment shows. One adds to 5 ml. of virulent suspension with a titer of 1/8192, 1 ml. of aluminum hydrate suspension, and leave the mixture for 24 hours at 4°.

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1) The ovomucine has been prepared according to the method of A. Gottschalk and P. Lind (Brit, J. Exp. Path. 1949, 30, 85).
2) Filtrate obtained at the expense of cultures of the strain Z4N in semi-fluid media, and titeres at the point of its stretching into enzyme RDE, following the techniques used by E. Nihoul (C.R. Soc. Biol. , 1951, 145, 1891). The titer of the preparation used was 1/320.

The titer of the supernatant liquid separated then after centrifugation os not more that 1/64: almost all of the virus is therefore fixed on the mineral precipitate. But, the residue, contained in 2.5 ml. of buffered physiologic solution causes to appear after intraperitoneal injection, an exudate which examined after 48 hours is different form the exudate collected in the animal which has received the same quantity of aluminym hydrate, not only by its abundance but also by the extreme frequency of the polynucleus pycnosis.

We have already pointed out in our previous article that the pycnosis of polynucleus is not perceptibel until 24 hours at least after the intraperitoneal injection of virus. We have studied later if the effects of virus on leucocytes would not be perceptible more rapidly if the latter was injectied in the peritoneal cavity already containg leucocytes whose formation has been caused by a broth injection done 6 or 7 hours earlier. The only apparent modification to the test doen 2½ hours after inoculation of the virus, consists in an agglutination of the cells of the exudate, which to the fact that the influenzal virus agglutinates the white corpuscles as well as the red.

SUMMARY

(1) The use of cultures of Pfeiffer bacillus on solid media (blood-gelose) reveals much more than those cultures on liquid media (bouillon-blood) than has permited ud to make préviously, the favorable influence of the toxic effectd of the influenzal

virus on the infection by that bacteria. For the strain which we have used the dose of culture on gleose-blood which injected intraperitoneally, causes death by septecemia, is about $\frac{1}{4}$ culture in the test animal. On the other hand this fatal dose varies according to the animal between 1/250 and 1/4000 of culture, in the guinea pigs having received the same way a half hour earlier a dose of influenzal virus which although less than the fatal doses duffices to cause the toxic effects which we have described previously and which consist in the production of a very abundant serous exudate characterized by the frequency of leucocytic pycnosis images. On an average the variation of the doses of Pfeiffer bacillus respectively fatal for the "influenza" guinea pig amd for the test guinea pig is 1 to 240 at least.

(2) The considerable decrease under the action of the influenzal virus, of the fatal dose of Pfeiffer bacillus cannot be explained by the simple accumulation of the respective noxious action of the virus and the bacteria, but it is more attributable to a sesitization produced by the firsr in respect to the second. In effect, under the same experimental conditions, the fatal dose of a bacteria such as colon bacillus, is not a normal associate of the influenzal virus, appears the same in "influenza" guinea pigs and the test guinea pigs. Under the dame conditions the pneumococci kill the influenza guinea pig with a dose approximately 10 times less than the dose which kills the test guinea pig. For a hemolytic streptococci, the variation has been quite considerable- between 100 and 200. Among normal

associates of the influenzal virus, the Pfeiffer bacillus and the streptococci, appear therefore particularly suitable to obtain benefits from the toxic effects of the virus.

(3) The toxic effects produced by the intraperitoneal inoculation of influenzal virus in the guinea pig appear independent of the hemagglutinat properties of the virus. The toxic power is in effect more sensitive to heat than the hemagglutinat power; on the other hand, the toxci action of the virus is not affected by the previous mixing of the virus with ovomucine which , as it is known, reduces considerably the hemagglutinat power. This toxic action manifests itself again if the virus before being injected has been adsorbed over aluminum hydrate.

In conclusion, the favorable influence--well known in man--which influenza exerts on certain associated infections, reveals a toxic action of the influenzal virus. It is manifested without delay after injection of a massive dose in the guinea pig, not receptive to the influenzal infection. The increase of sensitivity under the toxic action of the virus is nil in regard to the colon bacillus, is moderate (10 times) with respect to pneumococci, but it is considerable in regard to the hemolytic streptococci (100 to 200 times) particularly with respect to the Pfeiffer bacillus (at least 250 times).

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